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Complete genome sequence of a novel partitivirus from a wild brassicaceous plant, *Arabidopsis halleri*

AUTHOR(S):

Kamitani, Mari; Okuno, Tetsuro; Kudoh, Hiroshi

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1 **Title**

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5 **Author list**

6 Mari Kamitani ^{1,2}

7 Tetsuro Okuno ¹

8 Hiroshi Kudoh ²

9 ¹ Faculty of Agriculture, Ryukoku University, Yokotani1-5, Seta Oe-cho Otsu, Shiga 520-2194,

10 Japan

11 ² Center for Ecological Research, Kyoto University, Hirano 2-509-3, Otsu, Shiga 520-2113,

12 Japan

13

14 **Corresponding author**

15 Mari Kamitani

16 Email: kamitani.mari.86a@kyoto-u.jp

17 Hiroshi Kudoh

18 Email: kudoh@ecology.kyoto-u.ac.jp

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Abstract

Two contigs with high similarity to partitivirus species were identified by *de novo* assembly of sequences obtained by the RNA-Seq on a wild brassicaceous plant, *Arabidopsis halleri* subsp. *gemmifera*. Here, we determined the full-genome sequence of a putative novel partitivirus. Excluding the poly-A tail, it consisted of two RNA genome segments of 1912 and 1769 bp, which predicted to encode a 585-amino-acid-long putative RNA-dependent RNA polymerase (RdRp) and a 487-amino-acid-long putative capsid protein (CP), respectively. Phylogenetically, this virus belongs to the genus *Alphapartitivirus* and is most closely related to *Raphanus sativus* partitivirus 1 reported from radish. We propose the name of the novel virus, *Arabidopsis halleri partitivirus 1* (AhPV1).

51 Knowledge on viruses in wild plants is quite limited, although wild plants may act as
52 reservoirs of crop diseases. Recently, increasing numbers of studies are trying to reveal plant
53 virus diversity in nature using data obtained from high-throughput sequencing technologies.
54 Partitiviruses are one of the most frequently identified viruses in such surveys [3, 7]. They
55 possess two essential dsRNA genome segments, RNA1 and RNA2, which encode RNA-
56 dependent RNA polymerase (RdRp) and capsid protein (CP), respectively [5]. Members of
57 the family *Partitiviridae* have been reported not only from plants, but also from fungi and
58 protozoa. Partitiviruses are known as cryptic viruses that cause few or no visible symptoms in
59 their hosts [5, 6]. In our previous study, we conducted virus survey in a brassicaceous plant,
60 *Arabidopsis halleri* subsp. *gemmifera* (*A. halleri*), using RNA-Seq with Illumina HiSeq 2500
61 and *de novo* assembly [2]. In total, 68 plants were sampled and each of the leaf samples was
62 barcoded with different index-sequence and sequenced using 1 lane of Illumina HiSeq 2500.
63 Infection of *Turnip mosaic virus*, *Cucumber mosaic virus*, *Brassica yellows virus* were
64 determined and in *de novo* assembly, we detected two novel sequences with high similarity to
65 partitiviruses [2]. The two sequences were also detected by RT-PCR. Both sequences had
66 open reading frames encoding a putative RdRp and CP, respectively and were always detected
67 simultaneously in the partitivirus-infected plants [2]. These sequences were detected from 56
68 plant-individuals in the examined 68 plants. In this study, we determined the full-length
69 genome sequence of the putative partitivirus by conducting a rapid amplification of cDNA
70 ends (RACE) analysis on the 5' and 3' ends of the genome segments. Total RNA was
71 extracted from the leaves of the virus-infected *A. halleri* plants using Maxwell 16 LEV Plant
72 RNA Kit (Promega, WI, USA). Reverse transcription (RT) was conducted with oligo-dT
73 primers and Superscript IV Reverse Transcriptase (Thermo Fisher Scientific Inc., MA, USA).
74 The 3' and 5' ends of the two genome segments were amplified using the SMARTer RACE
75 5'/3' Kit (Takara-bio, Japan) according to the manufacturer's instruction. The rest middle-part

of the two segments were also amplified using primers constructed based on the sequence determined by 3' and 5' RACE (Supplementary Figure 1). The whole-genome sequence was determined by Sanger sequencing (Eurofin Genomics, Luxembourg). Both strands of the AhPV1 ds-RNA genome were detected by strand specific RT-PCR (Supplementary Figure 2). These sequences were deposited as the complete genome sequence of AhPV1 in the National Center of Biotechnology Information (NCBI) GenBank database with accession numbers MT155793 and MT155794.

The two segments of the AhPV1 genome were 1912 and 1769 bp in length excluding poly-A tail and encoded putative RdRp and CP, respectively (Fig. 1). The lengths of the deduced amino-acid (aa) sequences of RdRp and CP were 585 aa and 487 aa, respectively. The lengths of the untranslated regions at 5' and 3' ends of the segments, respectively, were 77 bp and 77 bp in RNA1, and 115 bp and 190 bp in RNA2 (Fig. 1). A phylogenetic analysis using the deduced amino-acid sequence of RdRp of this virus and reported partitiviruses [5] indicated that this virus belongs to the genus *Alphapartitivirus* (Figure 2) and is most closely related to *Raphanus sativus partitivirus 1* (RsPV1), which has been reported from a brassicaceous crop plant. The aa sequence identity of RdRp between RsPV1 and AhPV1 was 80.6%. The sequences of untranslated region (UTR) of RNA1 from RsPV1 and AhPV1 also had high similarity (Supplementary Figure 3). The nucleotide identity between RsPV1 and AhPV1 was 61.0% (50 sites against aligned 82 nucleotide-sites) and 65.4% (53 sites against aligned 81 nucleotide-sites) for 5' UTR and 3' UTR, respectively. The conserved A/T-rich regions observed in the 3' UTR might contain a polyadenylation signal of these viruses. The CP aa sequence of RsPV1 has not been reported; however, the CP aa sequence of AhPV1 showed 32.7% identity with that of *Rosellinia necatrix partitivirus 2* (RnPV2). The identities of 5' and 3' UTRs between the viruses were 35.7% and 44.9%, respectively. Segmented viruses including partitivirus have the capacity to exchange their genome segments in co-infection through reassortment, which drives

rapid evolutionary-changes [4]. We compared phylogenetic locations of RNA1 (RdRp) and RNA2 (CP) of AhPV1 to analyse whether AhPV1 could be derived from reassortment among disparate species or not (Figure 2 and Supplementary Figure 4). In the phylogenetic tree of CP, four genera of partitivirus did not always form single clades as observed in a previous study [1]. Among partitiviruses whose CP sequences were reported, AhPV1 was most closely related to RnPV2 (Supplementary Figure 4). Therefore, phylogenetic locations of the two genome segments of AhPV1 were similar and no obvious evidence of reassortment was observed. Plant-infecting partitiviruses are known to be transmitted intracellularly during cell division and to persistently infect hosts. Horizontal transmission via vectors has not been reported, while vertical transmission through seeds has been reported widely [3, 8]. We determined the seed transmission rate of AhPV1 using 22 seedlings from the surface-sterilised seeds obtained from three AhPV1-infected wild *A. halleri* plants. The AhPV1 infection was detected in 16 out of 22 seedlings by RT-PCR using primers designed to amplify RdRp sequences; forward ATGAAGAACACCGTCGTTCTC, and reverse GACTTCAGTTTCCCGTCATAC. This result indicates that the seed transmission rate was 72.7%.

In summary, we characterized a putative novel virus from a wild brassicaceous plant, and it was considered to belong to the genus *Alphapartitivirus*. The criterion for species demarcation in the genus *Alphapartitivirus* is that the two species have less than 90% and 80% identity between the amino-acid sequences of their RdRps and CPs, respectively [8]. Considering this criterion, we regarded AhPV1 as a novel species of the genus *Alphapartitivirus*. Because the family *Partitiviridae* includes both plant and fungal viruses, improving our knowledge about these viruses is a promising way to understand the evolutionary relationships or horizontal transmission of viruses between plants and fungi.

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128 **Declarations**

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133 **Competing interests**

134 The authors declare that they have no conflict of interest.

135 **Availability of data and material**

136 The full-genome sequence of AhPV1 were deposited in the NCBI database with accession
137 numbers MT155793 and MT155794..

138 **Authors' contribution**

139 M.K. and H.K conducted field sampling. M. K. conducted the laboratory experiment and wrote
140 the manuscript. M.K., T.O. and H.K discussed the results. All authors approved the manuscript.

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Figure legends

Figure 1 Diagram of the genome organization of *Arabidopsis halleri* partitivirus 1(AhPV1) detected from *Arabidopsis halleri*

(A) Representative individuals of *Arabidopsis halleri* infected by the novel partitivirus without apparent symptoms under natural environments. (B) Schematic diagram of AhPV1 genome. The putative ORFs and untranslated regions were indicated by boxes and lines, respectively.

Figure 2 Phylogenetic location of *Arabidopsis halleri partitivirus 1* (AhPV1) based on RdRp aa sequences.

Phylogenetic location of AhPV1 (boxed) was shown. The phylogenetic tree was constructed by MEGA7 using maximum likelihood method based on the Le_Gascuel_2008 model. Corresponding viruses and the accession numbers in NCBI database are listed in Supplementary Table 1. Vertical lines correspond to the four genera of partitivirus and *Cryspovirus* was added to as a fifth genus of partitivirus. *Otarine picobirnavirus* and human Picobirnavirus were used as outgroups. Underlining indicates related *Alphapartitivirus* for which the CP sequence is unknown. Numbers beside the clades represent bootstrap values for the branches supporting monophyly of genera.

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>AhPV1 RNA1

TCAAAATATAGGAAGGGAACCTACAAAGCTTCCATCTTTCTCTATTTTCTCTCAACAGACATTCTGTTTCACAC
ACTA[ATG]AAGAACACCGTCGTTCTCGAACCATGCCATCGCTGGCCAGGCCCATTTATGGAGATACCGACCC
Annnealing site of 5'RACE primer (R) Annnealing site of PCR primer (F)
AGGTCGAAATCCAGCCTACCAGAGTACAGTAGACCACGCACTCAGGCGACTCCTCACAGCTGAAGAGTTCA
ACATTGTCGTCAATGGCTACCGACGTTCCCCCTTGGAAATGAAGACGCCCTAACCGCCGATATTGAAAAGCTCA
ACTCTGACTATCACCACGTCAATAAAGATGAGCATTACTACAAAGCTATTGAACATACAAAGAAATTGTTTAC
ACCAAAGGAGAAATTAAGACCCGTGCATTTTAATGATCTACGTCACTACCCATGGCAATTGTCAACGAGTATT
GGCGCTCCATTTCGCGACAAGCGAAAAGTGGAAGGATTATATTAATCAGAAGTATGACGGGAAACTGAAGTCT
AGAGACTTTAAAGACCTATTCAAAGAACTCATGGAGTTTCGCTTGAACCATACATGATCGATAGACGCTTAT
CAAAGCGTAACCTTCTACAATGAAATGTTCTACATTAATCGAATTAATATTCATCACATTAAAGATGGATGGACA
ACGAATCCAGCAGGACACGATTTACGTTACTGGCATACTGCACACGCAAGACAACACTTAGTTGAAGCCGG
AGACGAAGACAAAGTCCGACTAGTATTTCGGTGCACCTTCTACCTTACTAATGGCCGAGCTCATGTTTCATTTGG
CCGATCCAGACTAGTTTACTAGCACGTGGATCTTCTTCGCCAATGTTATGGGGCTACGAAACCACTACAGGGG
GATGGTCCCGGTTATACAACCTGGGCATATTCTGCCCTTCCCAGATTTCGGAGCCGTCGCTACCCCTTGATTGGAG
TAGATTTCGACAAAGACGCTCGACATACTGTAATCACAGACATACATGATTTAATCATGCGCCCAATGTTTCGAC
TTCAATTCAGGCTACCCCAACTATAATCAACCCAAGATCTAATCCAGACCCGCAAAGGCTGGAGAATCTAT
GGAATTGGATGAAGAATGCAATCCTAACGACCCCTCTGCTGCTGCCAGATGGGACGAGACTACAATTCCAAC
ATTCTGGAATTTATTTCAGGATACCTTTCAAACACAGATATTAGACTCAATGTATAATTGCGTCATGATATTTACCG
TTCTTTCAAGAATGGGATTTCGATTTAAACTCAGTTGCTATTAAAGTACAAGGAGATGACTCACTCATCTTACT
CAGCCACAGCTACACTTTTCTTGCAACATTCGTTTCTGACTACGTTTGCACACCACGCTGCCGTATATTTTCGGC
TCGACGCTGAACGTAAAGAAAAGCGAGCTTTTACCATCACTAGAAGACGCTGAAGTTTTCGAGATACAGAAA
TCATGGTATGATGCCATATCGTGAAGAACTTCAACTACTAGCAATGCTACGACACCCAGAAAGGACTGCCTC
ACTCTCAGCCCTCATGGCACGAAGCATCGGAATAGCATAACGCTAACTGCGGAAACTACACCCGTGTACACCA
CATCTGCGAGGATATCCACAATTACCTAAAAGGATTGGGGTTAAGCCAGACGCAATTTGGATTACCAAGGTGG
ATTAAGGTTTCGAAAGAACTACCTCCCTCTTATGAAGAAATCGACATCAGCCACTTCCCAACATGGCTAGA
GACCGTCGAACGCTTACTAGACCCCTCAAGACCTCTGCTTACCAACAAGCACTGGCCTACCACTCACTTT
Annnealing site of PCR primer (R) Annnealing site of 3'RACE primer (F)
TTCGGTATCCCCGGAGAGTCC[ATG]GATAGGACGTTATTAATATTTATTTTACGTTTCTGCCCTTTGTAACATA
AAATTTAAATAATAATAATAATATATAAAAAAAAAAAAAAAAA

>AhPV1 RNA2

TAAATAACTGGAGAAATTACTACCAATTTCAAATTTCCACGTTATAATTAACCCAACAGATATTCTGTTCCC
TTATATAGTCCCCACTCACCACAACAACCTGTCTACTTACA[ATG]TCGATGAAAAGAAAGTCAAGGCCTAGT
TCTTCAACAAAAGATTAGAGGTAGAAGACACCTATCTCAAACAATCTGGACTTGATTCAATGAATAAGCTC
GAACCAGTCGAGCAATCAAAAGACGAAGAGACTACCAAAGTCTCCATGCTCCCCACCGCTTCTACTGCTATA
Annnealing site of RACE primer (R) Annnealing site of PCR primer (F)
ATCGCCCCGCGTAAACTAAGTTCGGAAGATTTTAGCTCTAAACGTAAGCCGGATCAAACGTCGCTGTTAGC
CCATTCTTTGGGTTCTTAGGACCCACATCCTACACCCTACACAGGGCAGGCTCTCACACTACTACCCCTTCT
GCCACATGATGGACTACATTCTTCACTCTATCAATTCACCTCTCTGTGATAATTACTACTTCAAGAGAGAACT
CCAAACTACCAACCTTACATTCTCCGACTCTACTTCGGAGTTCTTTTCTGGGTTTCAGTGCTTGCAGCTGGAA
ATGATGTTTCAGGTCATTAATGACCTACACTACGATTTCTTGCAGCGTTTCTTAGACTGCAATCCTCTCGAGTCT
TTGGCCATTCCAGGCCCACTTCTCGGACTCTTCAAGACACTCTGCTCCTCCAGCCAGAGTTCCCACATTATG
GGAAAGTTTACCCCCGATTCCTGCGAGCCAGGACCAAGACGCCGAGACATGTTCTCAAAGAACGTCCCA
AGCGCCCAATTCTTACCCAACGTTCTGCTATTTTCGCACTATCCACCATCTCCACGGACTTTCTGAGGGTG
AACACCCGATTATCCCGAAAAGAAAAGACACATTCTGTCACTGAGGAGGCCAGTAACTTCGGTTTCAAG
GCCTTCGCGCTTTTCCAAACAGAATCCAGCGCATCGTTGGATTGTTAGCTCCCCCGCCCTCCAGTATCCCT
GTGAAGCCGACATGAAGATGAATGAAGCGTTTCGCTGAACGTTTCTATGATTTTGACTTTCTGCTTTAACGC
AGATGACAATCTCTCCACCATCACCAACTTCTCCACATGAGGAAAAGTATGGCTTGGTTCATCCGGGCCAA
GGAAGTCGCCTGCTCGGCCGCTAGATTCTTTTCAGACTCTGGCACTCTCGCCGACTGTTCTCCACACGGTCT
GGTCTCAAACCAGATCATTTGTTGCGATTACTCCTCCACCTGAGGAGACTTTTGTGATCCCCGCTTCTCCGCC
GATCCAAGAGCCCTCTATCCCTTCAGTTTCAAGCTGAAGAGACCCGCCACAACCTCCCCCACTTGCGGAA
GCTGCCGACGCCCTTCTCCAGACGCACATCCGGATTTTTCGGGAATACCCGTTGGCCGGAAACTTTGGTCAA
AAGACCGACGAATCAGGCCCTTTTGGGACATCAGGCCCATTTGGCTCCAGCCCCACCGACGACACCTCCTA
CCTCACCATCCCACCCATGGTCAAGGCAGCACTCATCGAGAAAGGCTCCAGCCGT[ATG]GAGTCATGCACTG
ACGAGACATCAGCCGCTGACCACCTTTTTTCTTTTCTAGTATCTCGGATTTAGAAAGCAGCAATTTTACAAA
AGCAGTCCCATTTCGGACTAGCTGCTTTTCTTACGCATTTGCGTTTGCTTTTCCCTTTTAGTATTCCTTCTTTA
TTACTAAATGTATAACTTAAATTAAGAAAAA

Supplementary Figure 1 Complete nucleotide sequence of the two genome-segments of AhPV1 and location of primers used to amplify the whole sequences.

Start codons and stop codons are marked in boxes. Primers used for 5'RACE or 3'RACE is indicated by double underlines. Shaded sequences represent PCR primers to amplify the rest middle-part of the segments. Bold "A" characters at 3'end of the segments represent sequences that are regarded as poly-A tails.

Supplementary Figure 2 Detection of both strands of AhPV1 dsRNA

(A) Schematic diagrams of ds RNA of AhPV1 RNA1 was shown. Arrows represents RT and PCR primers used for strand-specific RT-PCR. (B) Amplified fragments on 1% agarose gel were shown.

Comparison of 5' UTR (A) and 3' UTR (B) were shown. Common nucleotide-residue at each site is shaded by black. The sequence of RNA2 was not reported for RsPV1.

Supplementary Figure 4 Phylogenetic analysis of AhPV1 based on CP aa sequences.

Phylogenetic location of AhPV1 (boxed) was shown. The tree was constructed by MEGA7 using maximum likelihood method based on the Jones-Taylor-Thrntton model. Viruses were indicated by their abbreviation listed in Supplementary Table 1. Vertical lines and colours correspond to the genera. Numbers beside the clades represent bootstrap values for the branches.

Supplementary Table 1 List of the species* used in phylogenetic analysis

No.	Reference strain abbreviation	Species name	GenBank accession no.	GenBank accession no.
			dsRNA1(RdRP)	dsRNA2(CP)
1	AhPV1	<i>Arabidopsis halleri partitivirus 1</i>	LC151461	LC151462
2	RsPV1	<i>Raphanus sativus partitivirus 1</i>	KT285019	-
3	VfPV1	<i>Vicia faba partitivirus 1</i>	DQ910762	-
4	AfuPV1	<i>Aspergillus fumigatus partitivirus 1</i>	FN376847.3	FN398100.2
5	AhV	<i>Atkinsonella hypoxylon virus</i>	L39125	L39126
6	AoV	<i>Aspergillus ochraceous virus</i>	EU118277	EU118278
7	BCV1	<i>Beet cryptic virus 1</i>	EU489061	EU489062
8	BCV2	<i>Beet cryptic virus 2</i>	HM560703	HM560702
9	BfPV1	<i>Botryotinia fuckeliana partitivirus 1</i>	AM491609	AM491610
10	CanCV	<i>Cannabis cryptic virus</i>	JN196536	JN196537
11	CarCV	<i>Carrot cryptic virus</i>	FJ550604	FJ550605
12	CaRV1	<i>Colletotrichum acutatum RNA virus 1</i>	KC572132	KC572133
13	CCCV2	<i>Crimson clover cryptic virus 2</i>	JX971982	JX971983
14	CCRSAPV	<i>Cherry chlorotic rusty spot associated partitivirus</i>	AJ781401	AJ781402
15	CpCV1	<i>Chondrostereum purpureum cryptic virus 1</i>	AM999771	AM999772
16	CrV1	<i>Ceratocystis resinifera virus 1</i>	AY603052	AY603051
17	CSpV1	<i>Cryptosporidium parvum virus 1</i>	U95995	U95996
18	DCV1	<i>Dill clover cryptic virus 1</i>	KF484726	KF484727
19	DCV2	<i>Dill cryptic virus 2</i>	JX971984	JX971985
20	DdV1	<i>Discula destructiva virus 1</i>	AF316992	AF316993
21	DdV2	<i>Discula destructiva virus 2</i>	AY033436	AY033437
22	DpCV	<i>Diuris pendunculata cryptic virus</i>	JX156424	JX891460
23	FcCV	<i>Fragaria chiloensis cryptic virus</i>	DQ093961.2	DQ355440
24	FCV	<i>Fig cryptic virus</i>	FR687854	FR687855
25	FpV1	<i>Fusarium poae virus 1</i>	AF047013	AF015924
26	FsV1	<i>Fusarium solani virus 1</i>	D55668	D55669
27	FvBV	<i>Flammulina velutipes browning virus</i>	AB465308	AB465309
28	GaRV-MS1	<i>Gremmeniella abietina RNA virus MS1</i>	AY089993	AY089994
29	HetPV1	<i>Heterobasidion partitivirus 1</i>	HQ541323	HQ541324
30	HetPV2	<i>Heterobasidion partitivirus 2</i>	HM565953	HM565954
31	HetPV3	<i>Heterobasidion partitivirus 3</i>	FJ816271	FJ816272
32	HetPV7	<i>Heterobasidion partitivirus 7</i>	JN606091	JN606090
33	HetPV8	<i>Heterobasidion partitivirus 8</i>	JX625227	JX625228
34	HTCV2	<i>Hop trefoil cryptic virus 2</i>	JX971980	JX971981
35	OPV1	<i>Ophiostoma partitivirus 1</i>	AM087202	AM087203
36	PepCV1	<i>Pepper cryptic virus 1</i>	JN117276	JN117277
37	PepCV2	<i>Pepper cryptic virus 2</i>	JN117278	JN117279
38	PerCV	<i>Persimmon cryptic virus</i>	HE805113	HE805114
39	PmV1	<i>Primula malacoides virus 1</i>	EU195326	EU195327
40	PoV1	<i>Pleurotus ostreatus virus 1</i>	AY533038	AY533036
41	PsV-F	<i>Penicillium stoloniferum virus F</i>	AY738336	AY738337
42	PsV-S	<i>Penicillium stoloniferum virus S</i>	AY156521	AY156522
43	RCCV1	<i>Red clover cryptic virus 1</i>	KF484724	KF484725
44	RCCV2	<i>Red clover cryptic virus 2</i>	JX971978	JX971979
45	RHsdRV2	<i>Rhizoctonia solani dsRNA virus 2</i>	KF372436	KF372437
46	RHsV717	<i>Rhizoctonia solani virus 717</i>	AF133290	AF133291
47	RnPV1	<i>Rosellinia necatrix partitivirus 1</i>	AB113347	AB113348
48	RnPV2	<i>Rosellinia necatrix partitivirus 2</i>	AB569997	AB569998
49	RoCV1	<i>Rose cryptic virus 1</i>	EU413666	EU413667
50	RsCV1	<i>Raphanus sativus cryptic virus 1</i>	AY949985.2	DQ181926
51	RsCV2	<i>Raphanus sativus cryptic virus 2</i>	DQ218036	DQ218037
52	RsCV3	<i>Raphanus sativus cryptic virus 3</i>	FJ461349	FJ461350
53	SsPV1	<i>Sclerotinia sclerotiorum partitivirus 1</i>	JX297511	JX297510
54	SsPV-S	<i>Sclerotinia sclerotiorum partitivirus S</i>	GQ280377	GQ280378
55	UvPV1	<i>Ustilagoidea virens partitivirus 1</i>	KC503898	KC503899
56	UvPV2	<i>Ustilagoidea virens partitivirus 2</i>	KF361014	KF361015
57	VCV	<i>Vicia cryptic virus</i>	AY751737	AY751738
58	VdPV1	<i>Verticillium dahliae partitivirus 1</i>	KC422244	KC422243
59	WCCV1	<i>White clover cryptic virus 1</i>	AY705784	AY705785
60	WCCV2	<i>White clover cryptic virus 2</i>	JX971976	JX971977
61		<i>Human Picobirnavirus</i>	AB186898	-
62		<i>Otarine picobirnavirus</i>	JQ776552	-

*Informations on No. 4 - No. 60 are obtained from Nibert *et al.*, 2014.